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ORIGINAL INVESTIGATION

Differential effects of psychotropic drugs on microbiome composition and gastrointestinal function

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Abstract

Rationale Growing evidence supports a role for the microbiota in regulating gut-brain interactions and, thus, psychiatric disorders. Despite substantial scientific efforts to delineate the mechanism of action of psychotropic medications at a central nervous system (CNS) level, there remains a critical lack of understanding on how these drugs might affect the microbiota and gut physiology.

Objectives We investigated the antimicrobial activity of psychotropics against two bacterial strain residents in the human gut, *Lactobacillus rhamnosus* and *Escherichia coli*. In addition, we examined the impact of chronic treatment with these drugs on microbiota and intestinal parameters in the rat.

Results In vitro fluoxetine and escitalopram showed differential antimicrobial effects. Lithium, valproate and aripiprazole administration significantly increased microbial species richness and diversity, while the other treatments were not significantly different from controls. At the genus level, several species belonging to *Clostridium*, *Peptoclostridium*, *Intestinibacter* and *Christenellaceae* were increased following treatment with lithium, valproate and aripiprazole when compared to the control group. Animals treated with escitalopram, venlafaxine, fluoxetine and aripiprazole exhibited an increased permeability in the ileum.

Conclusions These data show that psychotropic medications differentially influence the composition of gut microbiota in vivo and that fluoxetine and escitalopram have specific antimicrobial activity in vitro. Interestingly, drugs that significantly altered gut microbial composition did not increase intestinal permeability, suggesting that the two factors are not causally linked. Overall, unravelling the impact of psychotropics on gastrointestinal and microbiota measures offers the potential to provide critical insight into the mechanism of action and side effects of these medications.

Keywords Psychotropics · Intestinal permeability · Gut microbiota · Diversity · Richness · Short-chain fatty acids · Antimicrobial

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Introduction

The burden of psychiatric disorders on society continues to grow, with estimates from the World Health Organization (WHO) suggesting that worldwide, 322 million and 60 million people are affected by depression and bipolar disorder, respectively (World Health Organization 2017). Treatment options used in the management of psychiatric disorders are often associated with metabolic side effects (Beyazyüz et al. 2013; Correll et al. 2015; Olguner Eker et al. 2017; Reynolds and Kirk 2010; Tschoner et al. 2007) and high non-response rates (Ackenheil and Weber 2004; Al-Harbi 2012; Hatta and Ito 2014; Nelson 1998). Over the last decade, a growing body of evidence has highlighted a significant role for the gut microbiota in interactions between the gut and the brain (Bercik et al. 2012; Collins et al. 2012; Cryan and Dinan 2012b; Dinan et al. 2013; Mayer et al. 2014; Mayer et al. 2015). Moreover,

alterations in this microbiota-gut-brain axis are associated with several behavioural and psychiatric conditions (Cryan and Dinan 2012a; Foster and McVey Neufeld 2013; Luna and Foster 2015). Strikingly, individuals with depression (Jiang et al. 2015; Kelly et al. 2016; Naseribafrouei et al. 2014; Zheng et al. 2016), bipolar disorder (Evans et al. 2017) and schizophrenia (Dinan et al. 2014; Schwarz et al. 2017) exhibit alterations in microbiota composition compared to controls. Moreover, recent data has shown that the transfer of microbiota from humans with depression into microbiota-depleted rats or mice induces a depressive-like phenotype, indicating that the gut microbiota may play a key role in the onset of depressive behaviour (Kelly et al. 2016; Zheng et al. 2016).

Despite substantial scientific efforts to unravel the effects of psychotropic medications in the central nervous system, the impact that these drugs have on gut physiology and microbiota composition is rarely considered. Some isolated studies have shown that antidepressants and antipsychotics possess a microbiota-targeted action. Indeed sertraline, fluoxetine and paroxetine are bactericidal against gram-positive bacteria such as *Staphylococcus* and *Enterococcus* (Ayaz et al. 2015b; Coban et al. 2009); however, these data are mostly reliant on in vitro microbiological studies and are not exhaustive. Regarding antipsychotics, it has been shown that chronic administration of the antipsychotics olanzapine and risperidone affects the gut microbiota composition in animals and humans (Bahr et al. 2015b; Davey et al. 2013; Davey et al. 2012; Kao et al. 2018; Morgan et al. 2014). A recent study has demonstrated that intake of atypical antipsychotics is associated with significant changes in microbiota composition (Flowers et al. 2017a), while larger cohort studies have suggested that the use of medications can alter the gut microbiota more generally (Falony et al. 2016; Maier et al. 2018). Behavioural responses to cocaine, another compound belonging to the class of psychotropics, have recently been shown to be affected by gut microbiota shifts in mice (Kiraly et al. 2016).

Importantly, psychotropic medications are often administered orally, thus the gut microbiota represents a plausible target for distal action of these drugs. To this end, we sought to examine the effects that commonly used psychotropics have on microbiota composition and intestinal permeability, which is closely regulated by the gut microbiota (Karl et al. 2017; Ott et al. 2017; Ulluwishewa et al. 2011). Furthermore, we assess the antimicrobial activity of these medications against isolated strains resident in the human gut, *Lactobacillus rhamnosus* and *Escherichia coli*. Unravelling the comparative microbiome and gastrointestinal actions of different psychotropics in vivo will provide new insight into the mechanism of these drugs and their side effects and may have a critical impact on future clinical practice and drug discovery efforts.

Materials and methods

Bacterial growth-inhibition assay

Lactobacillus rhamnosus 6118 was grown in anaerobiosis at 37 °C overnight in MRS broth (BD Difco Lactobacilli MRS Broth). *Escherichia coli* APC105 was grown shaking overnight at 37 °C in BHI broth (Oxoid™ Brain Heart Infusion Broth). Overnight cultures were resuspended in broth to an OD reading (optical density at 600-nm wavelength) of 0.1, which corresponded to their lag phase. Resuspended cultures were incubated with a range of drugs dissolved in sterile deionised water at different concentrations (100, 400 and 600 μg/mL). The OD of the bacteria was measured every hour for up to 7 h. The vehicle consisted of the dissolution medium only, and each curve was produced in triplicates.

Animals

Adult male Sprague Dawley rats ($n = 8$/group; 200–250 g on arrival) were obtained from Envigo, UK. They were housed two per cage and maintained under a 12-h light/dark cycle, provided with chow and water ad libitum. Rats in the same cage underwent the same treatment to avoid confounding factors such as coprophagy. Animals were acclimated to housing conditions for 1 week prior to experimental treatment. Experiments were conducted in accordance with the European Directive 2010/63/EU. Approval by the Animal Experimentation Ethics Committee of University College Cork was obtained before commencement of all animal-related experiments.

Drug administration

Each drug was administered for 28 days in drinking water or in the chow, and the administration continued throughout the behavioural assessment until the animals were culled (Fig. 1, Table 1).

The control group received a standard diet (Ssniff, SM Teklad Global 18% Protein Rodent diet, item no. S9912-S710) and drinking water. A second group received 6.38 mg/kg/day of escitalopram oxalate (5 mg/kg/day of free base) in drinking water and standard diet. A third group

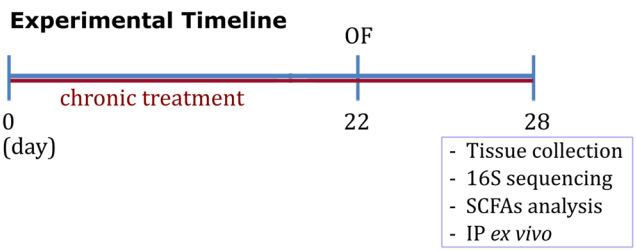
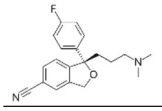
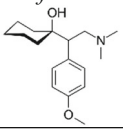
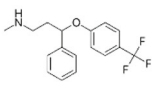
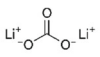
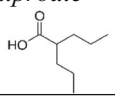
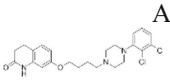


Fig. 1 Experimental timeline. OF open field, SCFAs short-chain fatty acids, IP intestinal permeability

Table 1 List of psychotropics employed in this study and their mechanisms of action

Drug	Class	Main medical uses	Mechanism(s) of action
Antidepressants:			
Escitalopram 	SSRI	Major depressive disorder, generalised anxiety disorder	↑ synaptic levels of 5-HT by blocking the reuptake of the neurotransmitter into the presynaptic neuron
Venlafaxine 	SNRI	Major depressive disorder, generalised anxiety disorder	↑ synaptic levels of 5-HT and NE by blocking the reuptake of the neurotransmitters into the presynaptic neuron
Fluoxetine 	SSRI	Major depressive disorder, generalised anxiety disorder	↑ synaptic levels of 5-HT by blocking the reuptake of the neurotransmitter into the presynaptic neuron
Other Psychotropics:			
Lithium 	Mood Stabiliser	Bipolar disorder, mood-stabiliser, major depressive disorder, schizophrenia	↑ release of 5-HT in the brain, interacts with NO signalling pathway in the brain, modulates glutamate levels, regulates mitochondrial function, lowers levels of inositol triphosphate
Valproate 	Anticonvulsant	Epilepsy, bipolar disorder, schizophrenia	Blocks voltage-gated sodium channels and ↑ brain levels of GABA. It has histone deacetylase-inhibiting effects
Aripiprazole 	Atypical Antipsychotic	Schizophrenia, major depressive disorder, bipolar disorder, obsessive-compulsive disorder	It is silent antagonist of some subpopulations of D_2 receptors but also a high-efficacy partial agonist of other D_2-receptor subpopulations. It has predominantly antagonist activity on postsynaptic D_2 receptors and partial agonist activity on presynaptic D_2 receptors. It is also a partial agonist of the D_3 receptor and the 5-HT_{1A} receptor

Upward arrow signifies increase, *SSRI* selective serotonin reuptake inhibitor, *SNRI* serotonin and norepinephrine reuptake inhibitor, *5-HT* serotonin, *NE* norepinephrine/noradrenaline, *NO* nitric oxide, *GABA* ɣ-aminobutyric acid, *D* dopamine

received 20 mg/kg/day of venlafaxine HCl in drinking water and standard diet. A fourth group received 10 mg/kg/day of fluoxetine HCl in drinking water and standard diet. A fifth group received 0.2% lithium-supplemented diet, corresponding to approx. 150 mg/kg/day, and hypertonic saline water (1.5% NaCl, in order to prevent lithium-induced ionic imbalance). A sixth group received 2% valproate-supplemented diet, corresponding to approx. 1.5 g/kg/day, and drinking water. A seventh group received 0.027% aripiprazole-supplemented diet, corresponding to approx. 20 mg/kg/day, and drinking water. The concentration of each drug in drinking water and in the chow was determined from the average daily water/food consumption and the average body weight per rat. These dosing regimens have been previously used in chronic behavioural and neurochemical studies in rats (Ariel et al. 2017; Kaminska and Rogoz 2016; Lyons et al. 2012; Monti et al. 2010; O'Leary et al. 2012; Segnitz et al. 2009; Sogaard et al. 2005; Vidal et al. 2010; Watase et al. 2007). The drinking bottles were protected from light and changed every second day, and the chow was stored at 4 °C during the experiment.

Open field (OF) test

To assess possible sedative effects of treatments, the animals were placed in an open arena brightly lit from above and the test was carried out as previously described (O'Mahony et al. 2014). Briefly, 30 min before behavioural testing, the animals were habituated to the room. The apparatus consisted of a white round arena with a diameter of 90 cm, brightly lit to 1000 lx. At the beginning of the test, animals were placed into the centre of the arena and allowed to explore for 10 min. After testing, the animals were returned to their home cage. The arena was cleaned with 70% ethanol between trials to ensure that no cue smell remained from the previous trail. Faecal output was manually scored. Total distance travelled was analysed using a tracking software system (Ethovision

XT 11.5, Noldus). None of the treatments at the doses tested affected the locomotor activity or the faecal output of the animals (Fig. S1).

Intestinal permeability

Freshly isolated ileal and colonic tissues were placed in Krebs solution and cut along the mesenteric border. Tissues were then mounted into the Ussing chamber apparatus (Harvard Apparatus, Kent, UK, exposed area of 0.12 cm^2) as previously described (Golubeva et al. 2017). Four kilodaltons of FITC-dextran was added to the mucosal chamber at a final concentration of 2.5 mg/mL; 200-μL samples were collected from the serosal chamber after 1 h and every 30 min for the following 3 h. FITC was measured at 485-nm excitation/535-nm emission wavelengths.

Microbiota composition and short-chain fatty acids analysis in the caecal content

Caecum was harvested and immediately snap-frozen and stored at − 80 °C prior to the analysis. DNA was extracted using the Qiagen QIAmp Fast DNA Stool Mini Kit coupled with an initial bead-beating step. The V3-V4 hypervariable region of the 16S rRNA gene was amplified and prepared for sequencing as outlined in the Illumina 16S Metagenomic Sequencing Library protocol. Samples were sequenced at Teagasc Sequencing Facility (TFRC, Moorepark) on the Illumina MiSeq platform using a 2 × 250-bp kit. Reads were assembled, processed and analysed following the pipeline, described in *Supplemental Methods*. Short-chain fatty acids (SCFAs) were measured by gas chromatography, using a Varian 3500 GC flame-ionization system fitted with a ZB-FFAP column. For construction of the heatmap, $\log_2$ ratios were calculated from group medians of highly abundant bacteria at the genus level using R (version 3.3.2) and R Studio (version 1.0.136).

Statistical analysis

Data are presented as mean + SEM. Intestinal permeability and in vitro data were analysed using mixed between-within subjects ANOVA followed by unpaired two-tailed *t* test. Body weight and organ weights were analysed using one-way ANOVA followed by Dunnett's test. 16S rRNA sequencing data was analysed using Kruskal-Wallis non-parametric test followed by Mann-Whitney *U* test and corrected for multiple comparisons using the Benjamin-Hochberg false discovery rate (FDR) method. Grubbs method was employed to test for any specific outliers (Grubbs 1950). Threshold for statistical significance was set at $p < 0.05$.

Results

Fluoxetine and escitalopram exert specific antimicrobial activity against two bacterial strain residents in the human gut

We tested the effects of psychotropic medications on growth of *Lactobacillus rhamnosus* 6118 and *E. coli* APC105 in vitro. These bacterial strains belong to two of the four dominant phyla of the mammalian gut (Firmicutes and Proteobacteria, respectively). Each drug was assessed at three different concentrations (100, 400 and 600 μg/mL). Growth curves were performed measuring the optical density (OD) at different time points. Growth of *L. rhamnosus* was completely inhibited by 400 and 600 μg/mL fluoxetine, while growth of *E. coli* was inhibited by 600 μg/mL escitalopram and by all three doses of fluoxetine (Fig. 2). Venlafaxine, lithium and valproate did not exhibit antimicrobial activity in vitro. Aripiprazole's antimicrobial activity was not assessed due to its tendency to precipitate with components of the broth, resulting in high risk of false positive data from OD measurements.

Administration of fluoxetine, lithium, valproate and aripiprazole significantly alters gut microbiota composition

Our in vitro results demonstrated that certain psychotropic drugs differentially modulate the growth of resident gut bacteria. To confirm this in an in vivo setting and to assess if any of the treatments induced changes in intestinal microbiota composition, we performed 16S sequencing of bacterial rRNA of the caecum content following chronic administration in rats. The sequencing revealed a significant increase in the bacterial richness and diversity of rats treated with lithium, valproate and aripiprazole as compared to the vehicle-treated group (Fig. 3a). Moreover, separation according to group was further illustrated through principal coordinate analysis (PCoA), with statistical support of the significant separation between escitalopram, venlafaxine, lithium, valproate, aripiprazole and the vehicle ($p < 0.05$, Fig. 3b).

At the phylum level, lithium induced a significant increase in *Actinobacteria* and a decrease in *Bacteroidetes*; valproate induced an increase in *Actinobacteria*, *Firmicutes* and a decrease in *Bacteroidetes*; fluoxetine induced a decrease in *Deferribacteres* and aripiprazole induced an increase in *Firmicutes* (Fig. S2, Table S1). At the family level, lithium, valproate and aripiprazole increased significantly the levels of *Peptostreptococcaceae*, *Clostridiaceae* and *Ruminococcaceae* (Fig. S3, Table S2). Other families were increased by the different treatments, while some of the less abundant families were decreased (refer to *Supplemental Material* for details). At the genus level of the most abundant

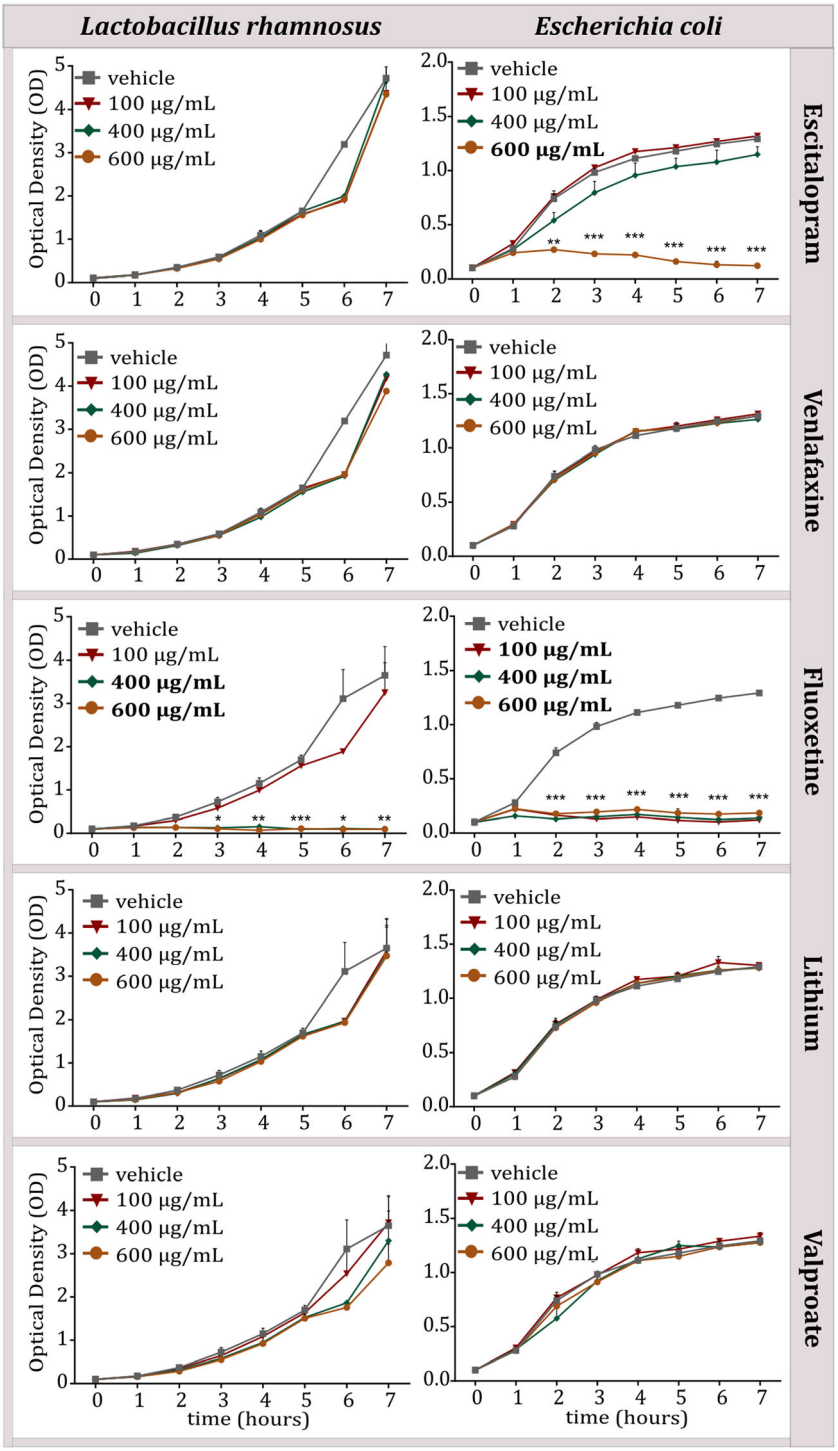


Fig. 2 Effect of psychotropic drugs on the growth of *Lactobacillus rhamnosus* 6118 and *Escherichia coli* APC105 in vitro. Escitalopram and fluoxetine have differential antimicrobial effects. In bold are bactericidal doses. *Statistics:* Data are expressed as mean + SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ ($n = 3$/group). Escitalopram effect on *E.coli*: $F_{(7;56)} = 491.682$, $p < 0.001$ for the effect of time, $F_{(3;8)} = 82.898$, $p < 0.001$ for the effect of treatment, $F_{(21;56)} = 59.87$, $p < 0.001$ for the time × treatment interaction. Fluoxetine effect on *L. rhamnosus*: $F_{(7;56)} = 42.467$, $p < 0.001$ for the effect of time, $F_{(3;8)} = 28.574$, $p < 0.001$ for the effect of treatment, $F_{(21;56)} = 15.439$, $p < 0.001$ for the time × treatment interaction. Fluoxetine effect on *E.coli*: $F_{(7;56)} = 188.805$, $p < 0.001$ for the effect of time, $F_{(3;8)} = 712.570$, $p < 0.001$ for the effect of treatment, $F_{(21;56)} = 176.477$, $p < 0.001$ for the time × treatment interaction. Data were analysed with a mixed between-within subjects ANOVA. F *F* statistic. Mean values in each time point were further compared to the vehicle with unpaired *t* test. Statistical outcomes for the *t* test are described in *Supplemental Material*

taxa, lithium increased the relative abundance of *Ruminococcaceae uncultured* and decreased the relative abundance of *Bacteroides* and *Ruminococcus* 1; valproate decreased the relative abundance of *S24-7 uncultbact* and increased the relative abundance of *Ruminococcaceae uncultured*, while aripiprazole decreased the relative abundance of *Ruminococcus* 1 (Fig. 4). None of the treatments significantly affected the genera *Lachnospiraceae uncultured*, *Akkermansia*, *S24-7 uncultbact* and *Oscillibacter*.

Interestingly, the relative abundance of minor genera including *Clostridium* sensu stricto 1, *Ruminiclostridium* 5, *Intestinibacter*, *Eubacterium coprostanoligens*, *Peptoclostridium*, *Eubacterium oxidoreducens*, *Christensenellaceae uncultured* and *Clostridia Family* XIII

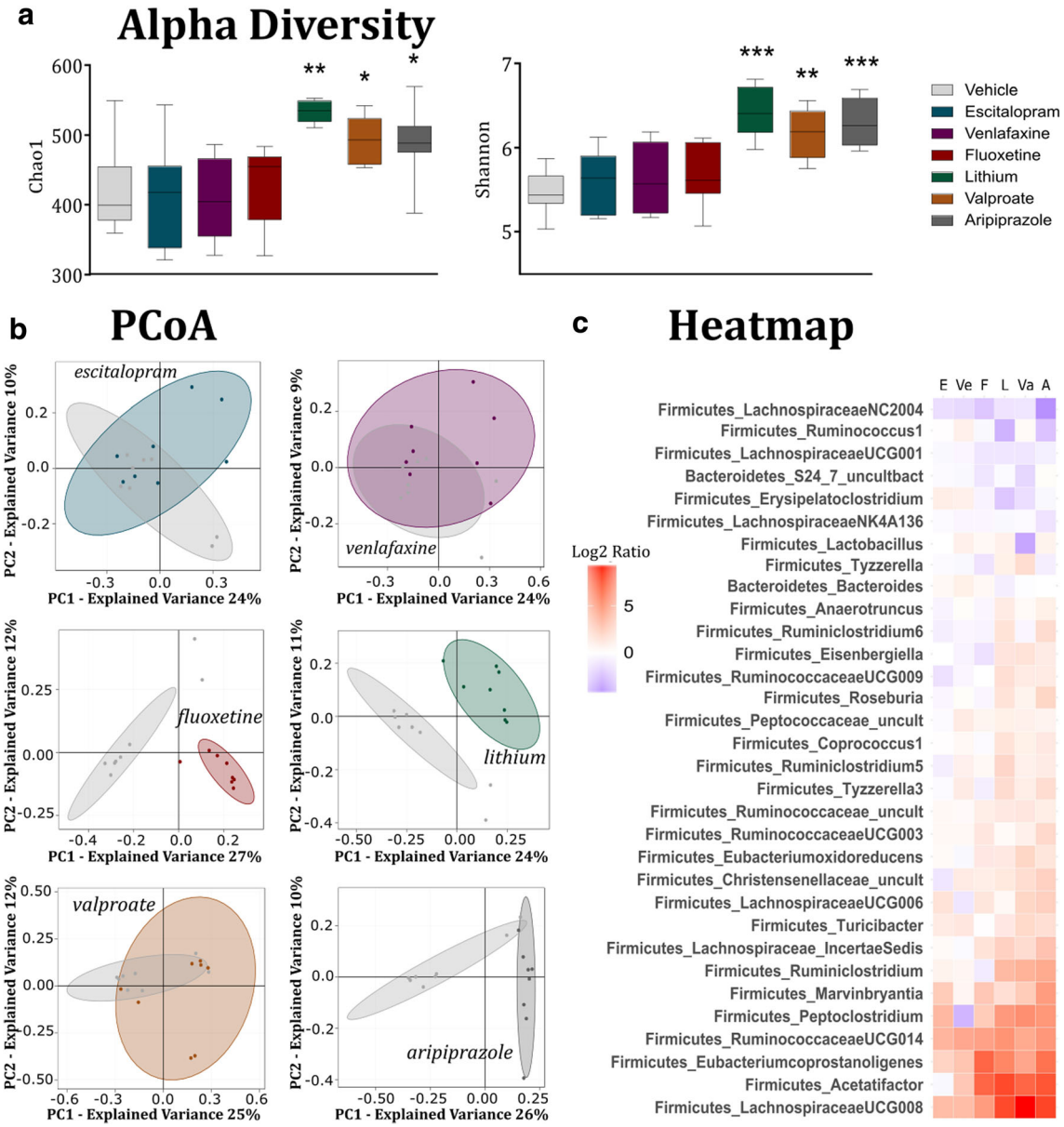


Fig. 3 Altered microbiota richness and diversity in psychotropic-treated animals as compared to vehicle-treated animals. a Alpha diversity. *Statistics*: Kruskal-Wallis test for Chao1 ($p = 0.000$) and Shannon ($p = 0.000$). Mann-Whitney *U* test for: *Chao1 index*: lithium $U_{(16)} = 6$, $p = 0.006$; valproate $U_{(16)} = 10$, $p = 0.021$; aripiprazole $U_{(16)} = 12$, $p = 0.036$. *Shannon index*: lithium $U_{(16)} = 0$, $p = 0.001$; valproate $U_{(16)} = 2$, $p = 0.002$; aripiprazole $U_{(16)} = 0$, $p = 0.001$. Data are expressed as median and min-to-max values. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ ($n = 8$/group). **b** Principal coordinate analysis of Bray-Curtis compiled distance matrix of all microbial relative abundances compared with the vehicle group (light grey ellipse). Escitalopram, venlafaxine, lithium, valproate and aripiprazole show significant variation from the vehicle (Adonis PERMANOVA $p < 0.05$). **c** Heatmap of $\log_2$ fold change ratio of medians at the genus level. Red indicates an increase and blue a decrease of taxa in the different treatment groups as compared to vehicle-treated rats. Microbial genera that were significantly different in at least one of the experimental groups compared to the vehicle were selected for the heatmap. E escitalopram, Ve venlafaxine, F fluoxetine, L lithium, Va valproate, A aripiprazole

was increased by lithium, valproate and aripiprazole administration (Fig. 5), while antidepressant administration did not influence significantly the abundance of these taxa. Among the antidepressants assessed, fluoxetine induced a marked depletion of the genera *Prevotella* 7, *Prevotella* 9 and *Succinivibrio* (Fig. 6).

Valproate and aripiprazole alter the levels of caecal short-chain fatty acids

A key function of gut bacteria is the catabolism of non-digestible dietary fibres resulting in the production of SCFAs, which in turn modulate a number of physiological

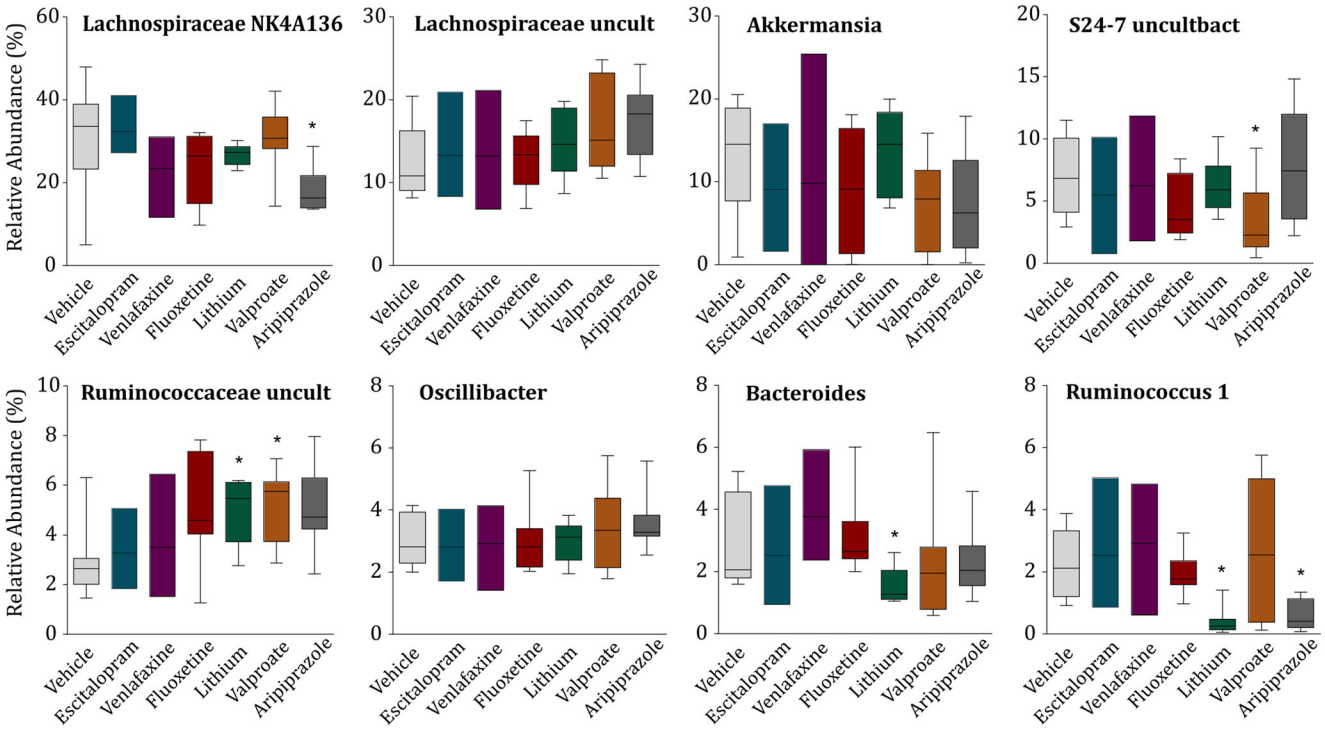


Fig. 4 Psychotropic drugs differentially affect bacterial composition at genus level of the most abundant taxa. Lithium increased the relative abundance of *Ruminococcaceae uncultured* and decreased the relative abundance of *Bacteroides* and *Ruminococcus* 1. Valproate decreased the relative abundance of *S24-7 uncultbact* and increased the relative abundance of *Ruminococcaceae uncultured*. Aripiprazole decreased the relative abundance of *Ruminococcus* 1. Data are expressed as median and min-to-max values. $*p < 0.05$ ($n = 8$/group). Data was analysed using Kruskal-Wallis non-parametric test followed by Mann-Whitney *U* test and corrected for multiple comparisons using the Benjamin-Hochberg false discovery rate (pFDR) method (refer to *Supplemental Material* for details on statistics)

processes (den Besten et al. 2013; Koh et al. 2016; Morrison and Preston 2016). We decided to examine the abundance of short-chain fatty acids (SCFAs) in the caecal content in response to psychotropic drugs. Valproate administration induced a significant decrease in the levels of propionate and butyrate while augmenting the levels of isovalerate. Aripiprazole administration induced a significant increase in the levels of acetate and isovalerate (Fig. 7). Two other SCFAs, valerate and isobutyrate, were also quantified and were not affected by any psychotropic treatment (see Fig. S4).

Escitalopram, venlafaxine, fluoxetine and aripiprazole administration increases ileal but not colonic permeability

We assessed paracellular intestinal permeability of treated animals in both ileum and colon tissues ex vivo. Among the drugs tested, escitalopram, venlafaxine, fluoxetine and aripiprazole administration significantly increased epithelial permeability in the distal ileum as compared to vehicle-treated animals (Fig. 8). None of the drugs at the doses tested affected permeability in the distal colon.

Discussion

There is an increasing emphasis on the interactions between gut microbiota and drug action across different therapeutic areas including oncology, cardiovascular medicine and even psychiatry. Here, we assessed whether orally administered psychotropics can affect microbial and intestinal function in healthy adult rats. Escitalopram and fluoxetine showed differential antimicrobial activity in vitro, whereas lithium, valproate and aripiprazole induced significant changes in gut microbiota composition and SCFA levels in vivo. Fluoxetine also induced minor but significant changes in bacterial genera in vivo. Escitalopram, venlafaxine, fluoxetine and aripiprazole increased the intestinal permeability in the ileum but not colon.

Limited studies have investigated the effect of psychotropic medications on growth of microbial strains. We found that both SSRI escitalopram and fluoxetine have diverse antimicrobial activity in vitro against *E. coli* and *L. rhamnosus*, two bacteria residing in the human gut (Thursby and Juge 2017; Walter 2008). These results are in line with previous work showing that SSRIs possess antimicrobial action in vitro especially against gram-positive bacteria such as *Staphylococcus* and *Enterococcus* (Ayaz et al. 2015a; Coban

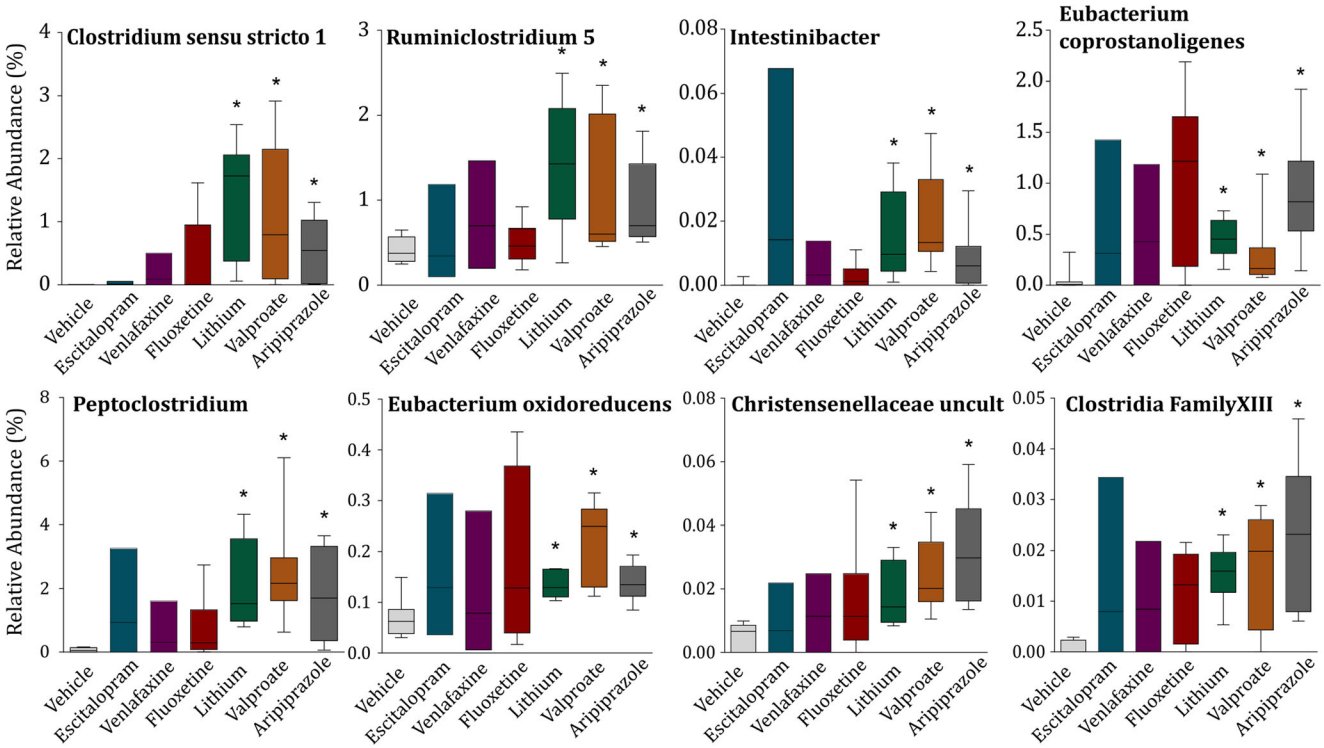


Fig. 5 Psychotropic drugs differentially affect bacterial composition at genus level of the less abundant taxa. Lithium, valproate and aripiprazole induced an increase in the relative abundance of *Clostridium* sensu stricto 1, *Ruminoclostridium* 5, *Intestinibacter*, *Eubacterium coprostanoligens*, *Peptoclostridium*, *Eubacterium oxidoreducens*, *Christensenellaceae* and *Clostridia Family* XIII. Data are expressed as median and min-to-max values. $*p < 0.05$ ($n = 8$/group). Data was analysed using Kruskal-Wallis non-parametric test followed by Mann-Whitney *U* test and corrected for multiple comparisons using the Benjamin-Hochberg false discovery rate (pFDR) method (refer to *Supplemental Material* for details on statistics)

et al. 2009). Moreover, the antimicrobial activity of some antidepressants has been previously confirmed by the synergistic effects of some SSRIs in combination with antibiotics, as well as their effects against some antibiotic-resistant bacteria (Bohnert et al. 2011a; Munoz-Bellido et al. 1996, 2000). Interestingly, the two drugs, despite both belonging to the same class of antidepressants, showed different effects, with fluoxetine having strong antimicrobial activity and escitalopram being a weak antimicrobial. A possible mechanism through which fluoxetine and escitalopram might inhibit bacterial growth is through their action as efflux pump inhibitors, which interferes with the normal functioning of the bacteria (Bohnert et al. 2011b). Intriguingly, among the drugs assessed in vitro, only fluoxetine induced overt shifts in gut microbiota composition in vivo. Moreover, differences in drug doses might occur in the two experimental settings, making it challenging to directly compare the experiments. These data highlight that some caution is required in extrapolating the results of in vitro assays to predict the effects of drugs on complex gut microbial ecology.

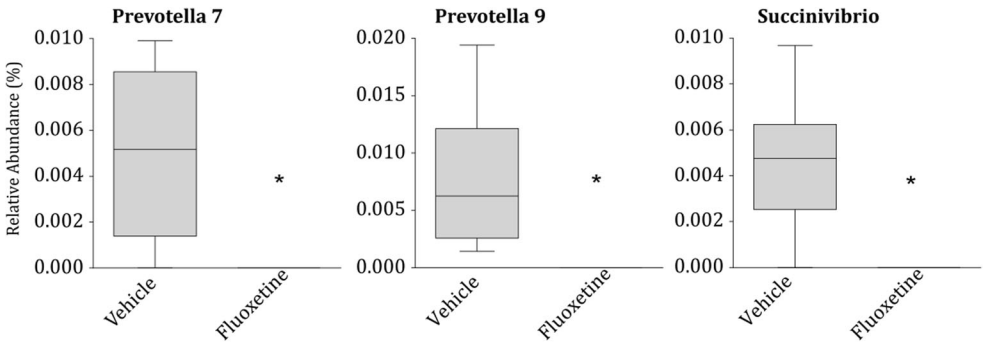


Fig. 6 Fluoxetine-sensitive genera in vivo. The genera *Prevotella* 7, *Prevotella* 9 and *Succinivibrio* were all depleted in the caecum of animals treated with fluoxetine. Data are expressed as median and min-to-max values. $*p < 0.05$ ($n = 8$/group). Data was analysed using Kruskal-Wallis non-parametric test followed by Mann-Whitney *U* test and corrected for multiple comparisons using the Benjamin-Hochberg false discovery rate (pFDR) method (refer to *Supplemental Material* for details on statistics)

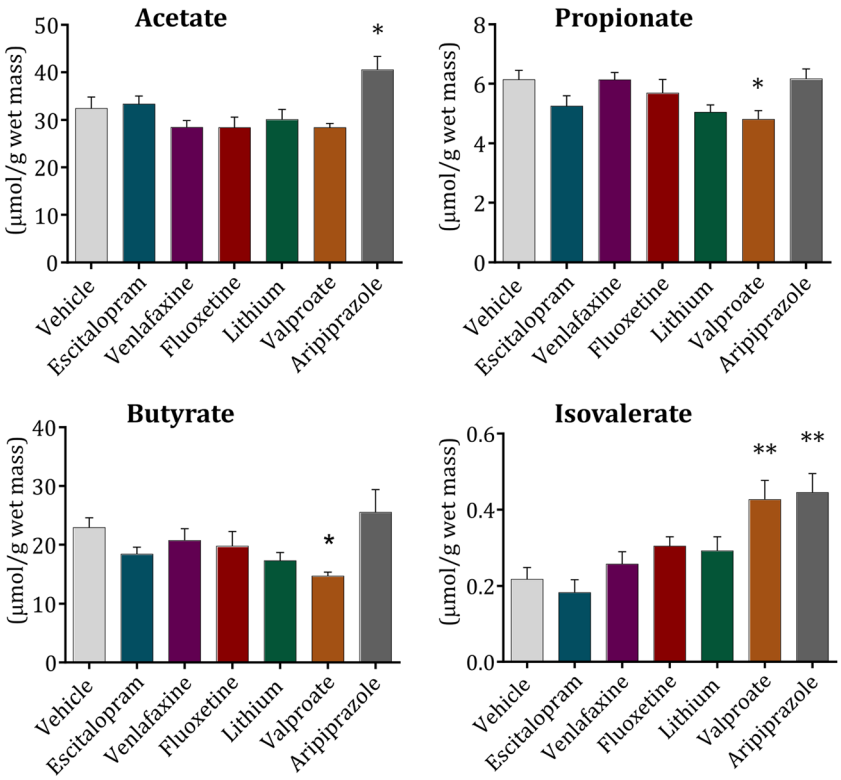


Fig. 7 Short-chain fatty acid (SCFA) caecal levels. *Statistics*: all SCFAs had $p < 0.05$ in one-way ANOVA. Acetate: Dunnett's t test $p = 0.035$ for aripiprazole vs vehicle. Propionate: Dunnett's t test $p = 0.029$ for valproate vs vehicle. Butyrate: Dunnett's t test $p = 0.042$ for valproate vs vehicle. Isovalerate: Dunnett's t test $p = 0.002$ for valproate vs vehicle; $p = 0.001$ for aripiprazole vs vehicle. Data are expressed as mean + SEM. $*p < 0.05$ and $**p < 0.01$ ($n = 8$/group)

The composition of the gut microbiota was substantially changed in vivo in lithium-, valproate- and aripiprazole- treated animals. Interestingly, the microbial shifts were often consistent across groups, with a significant increase in the relative abundance of minor genera (Fig. 6), while high abundant genera were generally not affected and were decreased in few instances (Fig. 5; i.e. *Lachnospiraceae NK4A136*, *Ruminococcus* 1, *Bacteroides*). Consistent with changes in bacterial genera, the alpha diversity was also augmented in animals treated with lithium, valproate and aripiprazole, suggesting an increase in microbial richness and diversity. Administration of the SSRI fluoxetine also induced changes in microbiota composition, specifically decreasing the genera *Prevotella* 7, *Prevotella* 9 and *Succinivibrio* (Fig. 6).

Some previous studies have shown that, among psychotropic medications, antipsychotics in particular exert an effect on the gut microbiota. Both atypical antipsychotics olanzapine (Davey et al. 2013; Morgan et al. 2014) and risperidone (Bahr et al. 2015a)-induced changes in gut microbiota composition in rodents and children, respectively. Chronic treatment with risperidone was associated with significantly lower ratio of Bacteroidetes:Firmicutes in healthy young males and with an increase in gut microbiota diversity when compared to control participants. Interestingly, we found the same trend in our animals treated with aripiprazole (a compound that belongs to the same therapeutic class of risperidone): at the genus level, 21 of the 26 genera belonging to Firmicutes were significantly increased and two *genera* belonging to Bacteroidetes were decreased. In addition, the microbial alpha-diversity of aripiprazole-treated rats was significantly increased, in line with data on risperidone-treated children. The results, however, are not always consistent. In a bipolar disease cohort, for example, treatment with atypical antipsychotics induced a decrease in microbial diversity, with the effect being present in females but not in males (Flowers et al. 2017b). In the same study, the bipolar cohort treated with atypical antipsychotics showed, at the microbiota genus level, a significant increase in *Lachnospiraceae* abundance and a significant decrease in *Akkermansia* (Flowers et al. 2017b). Also, a recent large-scale study in vitro looking at the effect of several non-antibiotic drugs on human gut bacteria in vitro found that *Akkermansia* was significantly more sensitive than all other strains to atypical antipsychotics (Maier et al. 2018). In contrast with these findings, treatment with the atypical antipsychotic aripiprazole did not affect the two aforementioned genera in our rats. The fact that antipsychotics cluster together on microbiome despite different chemical structures and CNS effects, implicates that direct bacterial activity may be part of their mechanism of action or at least their side effects.

An important aspect of this study would be to understand the biological and physiological relevance of the bacteria that were altered in response to psychotropic administration. Even though some of the affected *genera* have not been fully characterised, others have been previously associated to diverse conditions and are described in Table 2.

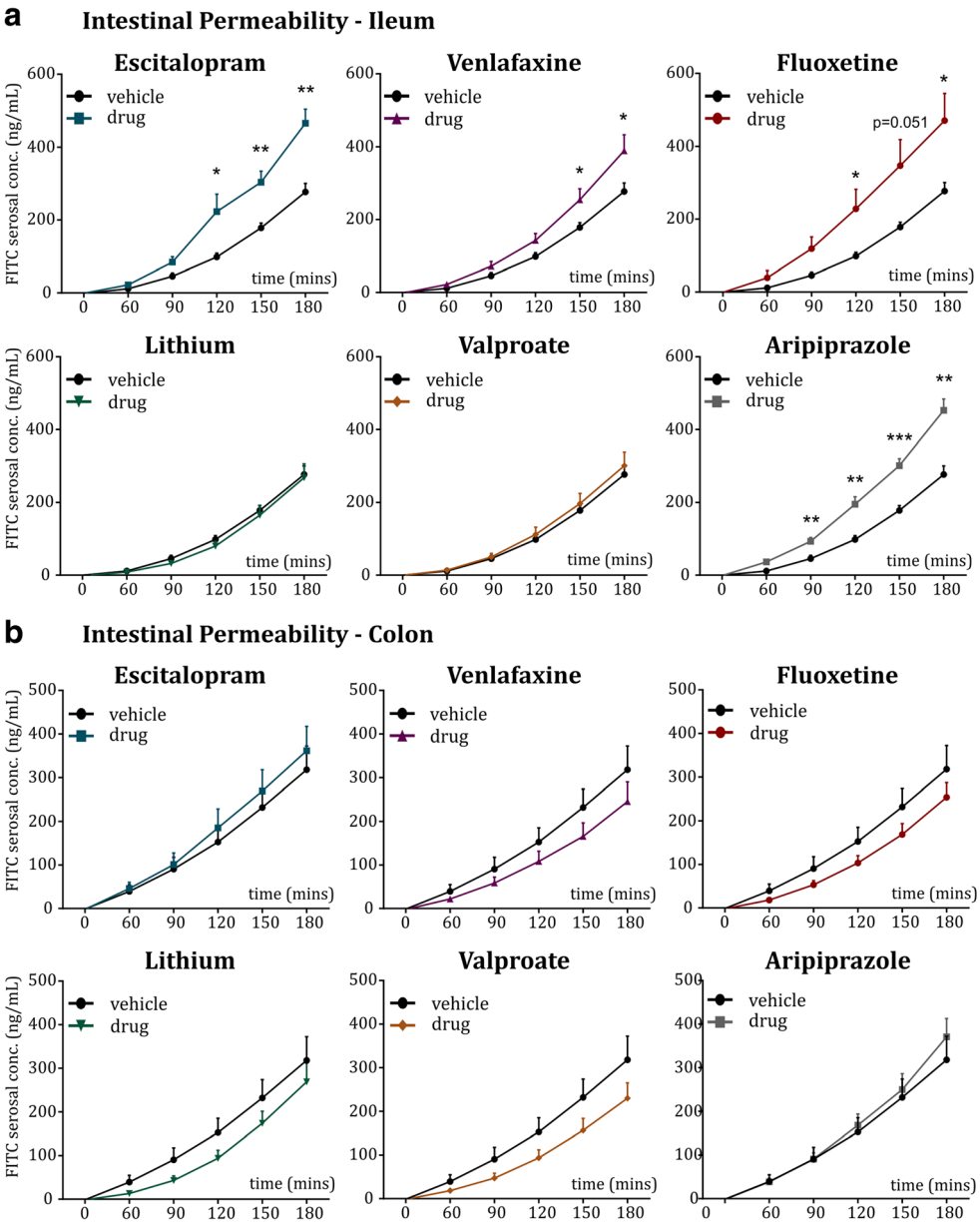


Fig. 8 Effect of drug treatments on epithelial permeability in small and large intestine. In the different panels, each treatment is compared to the vehicle. **a** In the distal ileum, escitalopram-, venlafaxine-, fluoxetine- and aripiprazole-treated rats showed a significant increase in FITC paracellular pemerability. **b** In the colon, none of the drugs induced significant changes in intestinal permeability. Data were analysed with a mixed between-within subjects ANOVA. *Statistics*: Distal ileum $F_{(5;235)} = 385.037$, $p < 0.001$ for the effect of time, $F_{(6;47)} = 4.181$, $p < 0.01$ for the effect of treatment, $F_{(30;235)} = 3.490$, $p < 0.01$ for the time × treatment interaction. Distal colon $F_{(5;235)} = 298.307$, $p < 0.001$ for the effect of time, but no effects for treatment or time × treatment interaction. F *F* statistic. Mean values in each time point were further compared to the vehicle with unpaired *t* test. Statistical outcomes for the *t* test are described in *Supplemental Material*. Data are expressed as mean + SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ ($n = 7$–8/group)

It is important to note that further work is required to measure the level of these drugs in the caecum, in order to clarify whether these medications reach the caecum at adequate concentrations and are not completely absorbed in the upper gastrointestinal tract. This investigation will further elucidate if the drugs are having a direct microbial effect or are affecting the gut microbiome through indirect mechanisms (such as the gut-brain signalling). Moreover, future studies might want to assess the microbiome-targeted effects of these medications at lower doses, such as those translational to a human setting.

While some knowledge existed of the impact exerted by antipsychotics on the gut microbiota, other classes of psychotropics (see Table 1) have not previously been investigated in vivo. Here, we show that chronic administration of the mood stabiliser lithium and the anticonvulsant valproate significantly affected the microbial composition and richness in rats. The increase in richness might be directly due to the effect of the drugs on the microbial stability and the presence of different bacteria competing for the same niche. This effect on richness might also be time- and dose-dependent. Principal coordinate analysis of Bray-Curtis (beta-diversity) showed that lithium, valproate and aripiprazole had a significant separation from the vehicle group. On the contrary, psychotropics belonging to the class of antidepressants (specifically escitalopram, venlafaxine and fluoxetine) did not markedly affect this aspect of microbial richness and diversity. Only fluoxetine clustered far from the vehicle group in the principal coordinate analysis of Bray-Curtis (Fig. 3b).

Short-chain fatty acids (SCFAs) are produced in the caecum by microbial fermentation (den Besten et al. 2013;

Table 2 List of bacterial *genera* that were altered by psychotropics and their associations with physiological/pathological conditions

Bacteria genus	Physiological/pathological conditions	References
Phylum Firmicutes		
Lachnospiraceae NK4A136	Associated with intestinal barrier function in mice with DSS-induced colitis. ↑ in mice fed a meat protein diet. ↓ in SAMP8 mice. Altered in statin therapy in mice. *Lachnospiraceae* strains restrict intestinal inflammation.	(Caparrós-Martín et al. 2017; Chen et al. 2017; Li et al. 2017; Xie et al. 2018; Zhan et al. 2018)
Ruminococcaceae uncult	*Ruminococcaceae* ↑ in mice fed a high-fat diet. ↓ in NAFLD patients. ↓ in CDI patients	(Kim et al. 2012; Raman et al. 2013; Schubert et al. 2014)
Clostridium sensu stricto 1	↑ in infants with high genetic risk of developing CD. ↑ in infants with IgE-mediated FA	(Ling et al. 2014; Olivares et al. 2014)
Intestinibacter	↓ in metformin therapy in patients with T2D	(Forslund et al. 2015; Wu et al. 2017)
Eubacterium coprostanoligenes	Cholesterol-lowering effects	(Freier et al. 1994; Li et al. 1998; Li et al. 1995; Ren et al. 1996)
Christensenellaceae uncult	↑ in longevity. Highly heritable. Associated to low BMI (anti-obesity)	(Goodrich et al. 2016; Kong et al. 2016; López-Contreras et al. 2018)
Phylum Bacteroidetes		
S_24_7 uncultbact	↑ in ageing	(Scott et al. 2017)
Bacteroides	HLA-B27 transgenic rats colonised with *Bacteroides* develop colitis and gastritis. Positively associated to immunoregulation in HIV-1 infection in humans	(Paquin-Proulx et al. 2016; Rath et al., 1996)
Prevotella 7 *Prevotella* 9	*Prevotella* species ↑ in healthy subjects who exhibit improved glucose metabolism following 3-day consumption of fibres	(Kovatcheva-Datchary et al. 2015)

Bacterial genera were selected from Figs. 4, 5 and 6 of the manuscript according to the following criteria: taxa that were significantly different from the vehicle in at least one treatment group. Among those taxa, *Ruminococcus* 1, *Clostridia Family* XIII, *Ruminiclostridium* 5, *Peptoclostridium*, *Eubacterium oxidoreducens* and *Succinivibrio* were not included in the table due to lack of information on their physiological role. *Upward arrow* signifies increase, *downward arrow* signifies decrease. *BMI* body mass index, *CD* coeliac disease, *CDI Clostridium difficile* infection, *DSS* dextran sulphate sodium, *FA* food allergy, *HIV* human immunodeficiency virus, *NAFLD* non-alcoholic fatty liver disease, *SAMP8* senescence-accelerated mouse prone 8, *T2D* type 2 diabetes.

Morrison and Preston 2016) and are key regulators of several host processes such as metabolism (De Vadder et al. 2014), behaviour (Schroeder et al. 2007) and CNS function (Erny et al. 2015; Huuskonen et al. 2004). With marked alterations present at the microbiota level, it was perhaps not surprising that changes in SCFAs occurred. Valproate and aripiprazole influenced SCFA abundance in the caecum, with acetate and isovalerate being increased by aripiprazole treatment and propionate, butyrate and isovalerate being differentially altered by valproate (Fig. 7). We next investigated whether changes in SCFA levels were associated with specific microbial taxa that are known producers of SCFAs. *Clostridium* spp., a known producer of the SCFA acetate (Koh et al. 2016), was found to be increased in aripiprazole-treated animals, while valproate-treated rats showed a decrease in *Bacteroidetes*, which might explain the reduction in propionate in this experimental group. However, in our study, the correlations between bacterial taxa and SCFAs were limited and sometimes discordant, for example, *Prevotella*, which is a producer of acetate (Koh et al. 2016), was decreased by aripiprazole administration. Therefore, the medications might have a different and direct influence on SCFA levels that are not mediated by the intestinal bacteria and still need to be teased apart.

The impact of psychotropic drugs on gut functionality is poorly understood. Thus, we assessed epithelial permeability in the small and large intestines and found that the three antidepressants escitalopram, venlafaxine and fluoxetine, together with the atypical antipsychotic aripiprazole, increased epithelial permeability in the ileum. Escitalopram, venlafaxine and fluoxetine share a common mechanism of action that is the blockade of the serotonin (5-HT) transporter (SERT) leading to increases in intrasynaptic 5-HT levels. SERT is not only present in the brain, it is also widely expressed on epithelial cells of the intestinal mucosa where it removes 5-

HT from the interstitial space following release by enterochromaffin cells (Chen et al. 2001; Chen et al. 1998; Coates et al. 2004; Wade et al. 1996). 5-HT is involved in the control of intestinal permeability (Bischoff et al. 2009; Haub et al. 2010; Yamada et al. 2003); thus, it is plausible to speculate that the changes observed in ileal permeability might be dependent on direct effects of the three antidepressants on SERT. In addition to the antidepressants, also the atypical antipsychotic aripiprazole increased permeability in the ileum; however, this effect is not SERT-mediated and may be due to its effects on other 5-HT receptors. On the other hand, aripiprazole induced a concomitant shift in gut microbiota and future studies are needed to determine if there is a causal link between microbiota and permeability in this specific treatment group. Interestingly, the action of psychotropics on intestinal permeability was region-specific, with the colon being largely unaffected.

Interestingly, some studies have demonstrated that the gut microbiota of depressed (Kelly et al. 2016; Naseribafrouei et al. 2014) and bipolar (Evans et al. 2017) patients has an abnormal composition. This suggests that the gut-targeted effects of psychotropics might be part of the mechanism of action of these medications; however, this needs to be further investigated and confirmed. In this vein, future studies examining the impact of these drugs on microbiota composition in animal models of mental disorders and subsequently on human cohorts are warranted.

The functional consequences of drug-induced microbiome changes maybe at multiple levels including drug efficacy, kinetics, side effects and safety. Regarding side effects, weight gain has been the most studied in the context of the microbiome due to the relationship between microbiota composition and obesity (Torres-Fuentes et al. 2017) and appetite regulation (Cani et al. 2009; van de Wouw et al. 2017). Indeed, weight gain induced by antipsychotics (including olanzapine and risperidone) can be modulated by targeting the microbiome with antibiotics and prebiotics (Bahr et al. 2015a; Bahr et al. 2015b; Kao et al. 2018; Morgan et al. 2014). In a recent meta-analysis, all of the antidepressants tested here increased weight gain in a population cohort (Gafoor et al. 2018), suggesting a dissociation between their effects on microbiome and bodyweight per se.

In conclusion, the present study demonstrates that psychotropic medications differentially affect gut microbiota composition and intestinal permeability in healthy adult rats. Interestingly, such changes do not parallel with the impact of these drugs on in vitro isolated bacterial strains or on intestinal permeability per se. Together, these data highlight the importance of investigating the impact of drugs used for the treatment of psychiatric disorders on microbiota-gut-brain axis function.

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Compliance with ethical standards

Experiments were conducted in accordance with the European Directive 2010/63/EU. Approval by the Animal Experimentation Ethics Committee of University College Cork was obtained before commencement of all animal-related experiments.

Conflict of interest All other authors declare that they have no conflict of interest.

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